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PRODUCTION OF ORGANIC ACIDS FROM CARBOHYDRATES BY FERMENTATION

A DIGEST OF THE LITERATURE

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INTRODUCTION

Fermentations resulting in the production of organic acids were in use long before the dawn of civilization. The herdsman who preceded the agriculturist in human chronology made lactic acid when he allowed his milk to sour for the production of curds or cheese, and housewives from the earliest days have depended upon this acid for the characteristic taste of sauerkraut and sour pickles. It is not unreasonable to assume that acetic acid also was known to the prehistoric wine maker. Butyric acid, the ingredient responsible for the odor of rancid butter, may very possibly share priority with the two first mentioned. Many other organic acids, such as the fruit acids, have also been known by their effect, if not by name, for centuries; but these do not come within the scope of this circular, as microorganisms play no recognized part in their production.

Regardless of the history of the various biochemical processes for the manufacture of organic acids, such processes have recently come to play a very important rôle in chemical industry. Within the last 30 years large industries have been based on such biological activities as the formation of acetic acid from alcohol by *Mycoderma aceti* and the formation of citric acid by *Aspergillus niger* when grown on sucrose solutions. These are only two of a number of examples of this type of reaction, the development of which is yet in its infancy.

In the study of fermentation reactions in the color and farm waste division of the Bureau of Chemistry and Soils, a large file of references on this subject is being built up, and the authors have re-

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ceived many requests for much of this material. For this reason, it is felt that it would be desirable to point out the sources to which the student of fermentations must turn for information and guidance. The material assembled in this circular, with the exception of that covering the manufacture of gallic acid from tannin, a glucoside, deals only with the production of organic acids from carbohydrates, and references to their direct formation from other substances, such as nitrogenous matter and alcohol, have been given only in patent citations which might have a direct economic significance. The larger part of the work thus excluded deals with the production of acetic acid from alcohol, a subject already exhaustively treated in various bulletins and monographs. No attempt has been made to deal with food materials, such as vinegar and sauerkraut, in which the production of an organic acid plays a predominant part, for such products have been so completely described elsewhere that reference to them is unnecessary.

Responding to the great interest in fermentation chemistry, several volumes which deal with this subject have recently been published. As the reader will find in them a fairly complete bibliography, no attempt has been made to repeat these references in this circular, but rather an effort has been made to mention outstanding articles on each subject and then point the way to sources from which fuller information may be obtained. The patent literature given here will, however, be found fairly complete in its record of United States and foreign patents. A careful search has been made of the files in the Patent Office, and all the fermentation patents found there have been given under the head of the product with which they deal.

In general, the statements covering the various acids have been confined to the barest outlines, except in the case of such products as citric acid, which have not received adequate treatment in the literature now available. In those instances a more detailed exposition of the subject matter has been given. It is the hope of the authors that the material included in this circular will be of assistance to others planning investigations in this field.

ACETIC ACID

Despite the fact that acetic acid, as vinegar, has been known for thousands of years, it was less than 100 years ago that Kützing (*63*)[1] first definitely demonstrated that it was formed by the oxidizing action of certain microorganisms on alcohol.

Acetic acid is found almost universally among the products of bacterial fermentations. It is a substantial part of the final products of the butyric, lactic, and propionic fermentations and is always present in varying quantities in alcoholic fermentations induced by bacteria. The reason for this widespread occurrence is apparent when the variety of transitory intermediates formed in various fermentations from which acetic acid might arise is considered. For a fairly satisfactory bibliography of the scattered literature the interested reader is referred to the index of Fulmer and Werkman (*47, p. 111–118*).

[1] Italic numbers in parentheses refer to Literature cited, p. 24.

Notwithstanding the great progress made in the past decade in the manufacture of anhydrous acetic acid by synthetic methods, considerable work has been done with a view to utilizing the activity of certain organisms in transforming cellulosic materials into acetic acid and ethanol. Thus Langwell has developed processes which have aroused considerable interest, in which crude manure cultures of thermophilic bacteria attack waste cellulose, forming acetic and butyric acids and ethanol. (See patents.) The procedures outlined in the Langwell patents are reported to be under commercial operation, and apparently the production of acetic acid in such a manner is economically sound at present.

Viljoen, Fred, and Peterson (*97*) have isolated cultures of thermophilic bacteria which destroy cellulose at 65° C., forming acetic acid. The quantity of cellulose destroyed in 1 to 5 per cent suspensions ranged from 70 to 95 per cent, 50 to 55 per cent of this being accounted for as acetic acid. Recently Scott, Fred, and Peterson (*85*) have reported an investigation in which cellulose was fermented at 55° to 65° C. by enrichment cultures of thermophilic bacteria, acetic acid constituting 45 to 65 per cent of the end products.

The work of Norbury and his associates on the production of ethanol, acetone, and acetic acid must also be mentioned. (See patents.) These investigators found that *Bact. acetoethylicus* fermented hydrolyzed straw, grasses, etc., producing substantial quantities of acetic acid.

The thermophilic fermentation of cellulose-containing materials offers a field of investigation which should prove of much value. At present our knowledge of cellulose-destroying organisms and of methods for controlling or regulating their activities is decidedly limited, and the situation with regard to these fermentations is in much the same state as that of, for example, the lactic acid fermentation in 1870. A thermophilic fermentation of cellulose which could be as well controlled as those now used for the production of lactic acid or butanol and acetone would prove of extreme economic importance.

BUTYRIC ACID

The butyric acid fermentation was first recognized as a definite microbial phenomenon by Pasteur (*77*), who described it as an anaërobic process. Various organisms, all rather closely related, were held responsible for the formation of the acid from sugar. Baier (*2*) differentiated at least six groups of organisms as well as many subgroups which were capable of bringing about a butyric fermentation. Of these Winogradsky (*116*) and Fitz (*41*, *42*) found the *Bacillus butyricus* and the granulobacter groups to be the most important. The classification of butyric acid-forming bacteria is incomplete, but reference may be had to that proposed by Bredemann (*13*).

Butyric acid formation in fermentation processes is generally accompanied by other reactions producing a variety of substances. For example, Buchner and Meisenheimer (*14*) reported the following as a typical fermentation of glucose by *Bacillus butyricus* Fitz: 100 grams of glucose with nutrient salts and calcium carbonate gave 0.7 gram butanol, 2. 8 grams ethanol, 1.6 grams hydrogen, 3.4 grams

formic acid, 10 grams lactic acid, 7.5 grams acetic acid, and 26 grams butyric acid. Kirov (*58*) obtained somewhat similar results with two types of butyric bacteria isolated from molasses. He found the fermentation of sugars by these organisms to be characterized by the formation of substances other than butyric acid, such as acetic and lactic acids.

Considerable work has been done in attempts to elucidate the intermediate steps involved in the formation of butyric acid and the compounds usually accompanying it by the fermentation of sugars. From the data so far accumulated it may be stated that methyl glyoxal, resulting from fission of the hexose molecule, is first formed, then broken down to acetaldehyde and formic acid, and the latter is further split to carbon dioxide and hydrogen. The aldehyde gives rise to acetic acid and ethanol and may also condense to aldol, from which butyric acid, β-hydroxy butyric acid, butanol, and acetone are formed. The lactic acid arises directly from methyl glyoxal. For a review of this work the reader is referred to the literature which has recently been compiled (*83, p. 72–80; 93, p. 142–152*).

The butyric fermentation, so far as is known, has never been applied industrially, at least on a tonnage scale. The patent literature describes several processes that may be of industrial promise, although in most cases the impression is gained that the work has been decidedly empirical. It would appear that commercial exploitation of any fermentation process for producing butyric acid must depend very largely upon the development of satisfactory means for the separation and recovery of other products of economic importance.

CITRIC ACID

The history of the mycological production of citric acid begins in 1893, with the report of Wehmer (*105, 106*) on its formation from sucrose by a species of fungus, to which he assigned the name Citromyces. He later reported that *Penicillium luteum* and *Mucor piriformis* also produced citric acid (*108*). During the last 20 years a great deal of work dealing with this fermentation has been recorded in the literature. Only the more important papers will be mentioned here. For fairly complete references the reader is referred to the bibliography compiled by Fulmer and Werkman (*47, p. 129–131*).

The first important milestone in the development of the citric acid fermentation was the work of Thom and Currie (*95*), by which they overthrew the idea, then current, that the black Aspergilli were exclusively oxalic acid formers. After this, Currie (*28*) undertook a classic investigation of the factors controlling the production of citric acid by a selected strain of *Aspergillus niger*. By using proper concentrations of sucrose (around 15 per cent), supplying the nutrient nitrogen in the form of low concentrations of ammonium nitrate, and adjusting the initial pH of the culture solution to 3.5 by addition of hydrochloric acid, Currie was able almost completely to suppress the formation of oxalic acid and obtained a rapid fermentation, with little loss of citric acid through consumption by the organisms, as long as any sugar remained in the substrate.

Most of the recent literature, aside from patents, deals largely with factors influencing the fermentation and with attempts to throw some light on the mechanism of the reactions involved in the interesting transformation by which the branched-chain compound is produced from sugars in which no such structure exists. Mazé and Perrier (*67*) were apparently the first to offer a theory to account for the reaction. They assumed the acid to be formed at the expense of albuminoid substances built up from the sugar and the nitrogenous nutrient material, with a continuous regeneration of the albuminoid. Buchner and Wüstenfeld (*15*) suggested that parasaccharinic acid was first formed from the sugar and then underwent a series of rearrangements, finally giving citric acid. In 1909, Herzog and Polotzky (*56*) observed that citric acid was formed from 3 and 5 as well as from 6 and 12 carbon sugars, thus making it necessary to introduce a condensation reaction into the process if the saccharinic theory was to hold. About this time, Euler (*39, v. 3, p. 182–183*) suggested that the sugars split, forming acetaldehyde, three molecules of which condense and are then oxidized to citric acid. Raistrick and Clark (*79*) have proposed a mechanism in which β- γ- diketo adipic acid is formed and is then hydrolyzed to acetic and oxalic acids, the former being oxidized to make another molecule of the latter. Oxal-acetic acid, occurring as an intermediate in this oxidation, was held to be the material from which citric acid was formed. Butkewitsch (*16, 17*), who has done considerable work on the citric acid fermentation, following the theory that gluconic acid was the first step in the process, thought that it might be possible for the gluconic acid to be transformed to glucuronic acid. This was then pictured as undergoing an inner aldol condensation with the formation of a 5-membered ring, the carboxyl group being without the ring (*18*). The condensation was supposed to take place between the aldehyde carbon and the carbon atom in the delta position to it, so that rupture of the ring with subsequent oxidation would give rise to citric acid. Wehmer (*110*) pointed out that calcium gluconate was converted into citric acid by *Aspergillus niger* and concluded that it was possible that the degradation of glucose by that organism might proceed from glucose to gluconic acid to citric acid to oxalic acid and eventually to carbon dioxide. Franzen and Schmitt (*44*) modified the old parasaccharinic acid theory in that they suggested saccharic acid as the chief intermediate product. This acid was supposed to undergo a further transformation into β- γ- diketo adipic acid, which then underwent a benzilic acid rearrangement by which citric acid was formed. In a series of papers Challenger and his associates (*24, 25, 101*) considerably strengthened the hypothesis of Franzen and Schmitt (*44*) by reporting the isolation of saccharic acid from cultures of *A. niger* on glucose. They also obtained citric acid by growing the same organism on solutions of potassium hydrogen saccharate and found that calcium gluconate was transformed to saccharic acid and also to citric acid. Kostytschew and Tschesnokov (*62*) expressed the opinion that the formation of citric acid is definitely related to the nitrogen metabolism of the organism, based on the assumption that the formation of nucleic bases and amino acids proceeds through the synthesis of the nitrogen-free framework with subsequent incorporation of the nitrogen.

They hold that if nitrogen is lacking, the last step is repressed, and the mother substances of the amino acids are transformed into citric acid by reactions which are not yet clear. Bernhauer (*8*) attempted to fix acetaldehyde in the course of citric acid fermentations and succeeded in obtaining a quantity equivalent to 0.6 per cent of the sugar consumed. He concluded that there is no relation between the occurrence of acetaldehyde and the rearrangement processes by which the citric acid is formed. Recently Bernhauer (*9*), as a result of experiments with 26 strains of molds, expressed the opinion that saccharic acid is not an intermediate product in the formation of citric acid.

Amelung (*1*), in 1927, reported the results of a large number of experiments in which he cultivated varieties of Aspergilli on 3, 4, 5, 6, 7, and 12 carbon sugars, and obtained the maximum yield of citric acid from sucrose. This is in agreement with the results of Molliard (*70*) and also of Bernhauer (*6*, *7*). The latter found that inulin and fructose approached sucrose as good carbon sources for the acid, and the yields with these three compounds were much greater than those obtained when other sugars were employed. As the yields from the fructose fragment of sucrose were higher than those from fructose itself, Bernhauer is of the opinion that the structure of the fructose residue of the sucrose molecule has special significance in the formation of citric acid. The fact remains that the mechanism for the mycological production of citric acid is still very obscure, and although considerable data have been accumulated, no hypothesis yet available satisfactorily accounts for all the observed facts.

Production of citric acid by mold fermentation of sucrose was attempted on an industrial scale in Germany more than 30 years ago (*54*), but so many difficulties were encountered that the process was unable to compete successfully with that based on recovery of the acid from citrus fruits, and was soon abandoned. Although much information is available in journal and patent literature, it is only within the last five years that production of citric acid by fermentation has taken a prominent place in chemical industry. The successful exploitation of this process has been accomplished through intensive research, and methods developed for carrying it out on a large scale consequently have been kept as closely guarded trade secrets. The process undoubtedly consists of a shallow-pan fermentation of sucrose by a strain of *A. niger*, and the reaction is completed in probably less than nine days. The quantity of fermentation citric acid produced can not be learned from the Department of Commerce, as the production is limited to one company, but unofficial estimates range from 2,500 to 5,000 tons per year. Its volume may be realized from the fact that this country now is not only independent of foreign citric acid but is actually exporting it to Europe (*92*). The industrial production of citric acid from sucrose by mold fermentation deserves to be ranked as one of the outstanding chemical achievements of the past decade.

FORMIC ACID

Formic acid occurs widely in a variety of anaërobic yeast and bacterial fermentations (*47*, *p. 138–140*), apparently arising directly from the 3-carbon intermediate, methyl glyoxal. In some cases,

notably in the fermentation of sugars by *Bacillus coli communis*, the acid is further broken down into carbon dioxide and hydrogen, and the hydrogen thus liberated apparently plays an important part in determining the extent to which other products, such as acetic and succinic acids and ethyl alcohol, are formed (*51*, *52*). That formic acid occurs and subsequently disappears in the fermentation of glucose by various yeasts is indicated by the work of Franzen and Steppuhn (*45*), but its significance is not clear. Zoller and Clark (*118*) have reported the formation of comparatively large quantities of the acid by organisms belonging to the dysentery group of bacteria, and they have expressed the opinion that the yields of acid were sufficiently high to be of some industrial significance. However, formic acid can be made so easily and cheaply by synthetic methods that it is doubtful whether any process based on the use of microorganisms will prove of commercial importance.

FUMARIC ACID

Formation of fumaric acid as a result of microbiological activity was first observed in 1911 by Ehrlich (*35*, *36*) who isolated this compound from glucose and fructose solutions on which *Mucor stolonifer* had been cultured. In 1918, Wehmer (*109*) announced the isolation of a strain of Aspergillus closely related to *Aspergillus niger* which produced fumaric acid from sucrose in quantities equivalent to 60 to 70 per cent of the sugar used. He gave the name *Aspergillus fumaricus* to this organism and proposed to oxidize to tartaric acid the fumaric acid produced by it, thus establishing a source for tartaric acid independent of the wine industry.

The subsequent history of this fermentation is extremely interesting. During the war Wehmer did no work with this organism, and it was kept in continuous culture on potato. When investigations were resumed in 1920, great variations in the yield of fumaric acid were found, as well as quantities of citric acid greater than in the earlier experiments (*111*, *p. 418–419*). Two years later reports of further work showed that in many cultures fumaric acid was entirely absent, whereas the yields of citric acid had increased (*111*, *p. 418–419*). Wehmer (*111*) again took up the problem, and in 1928 he reported that fumaric acid was formed by his organism only on rare occasions and then after long periods of culture. On the other hand, fairly large quantities of gluconic acid, together with varying quantities of citric and small quantities of malic acids, were produced. Experiments carried on with the object of restoring the ability of the organism to produce fumaric acid by varying the conditions of culture have almost completely failed to attain the desired result (*84*). However, Thies (*94*) has recently reported that inoculating glucose solutions with cultures developed on beer wort and carrying out the fermentation by aerating the substrate with oxygen caused the development of a heavy submerged growth. Analysis of the solution fermented in this manner revealed appreciable quantities of fumaric acid. Wehmer has termed the organism a "chemical riddle."

Takahashi and his associates (*88*, *90*, *91*) in the course of extensive investigations of the physiology of various Rhizopus species found

fumaric acid as a usual product of the fermentation activities of this genus of organisms. The quantity of acid produced was too small to be of any immediate industrial significance, at least under the conditions of culture used in their experiments.

The mechanism by which fumaric acid is formed from sugars by molds is quite obscure. Gottschalk (*49*, *50*) has reported that *Mucor stolonifer* transforms pyruvic acid to fumaric acid, but other investigators have questioned his results (*19*, *37*, *38*). Butkewitsch and Fedoroff (*19*, *20*, *21*) have carried out a series of experiments seeking to clarify the question of formation of the acid by *M. stolonifer*. They reported that with a relative deficiency of nitrogen and in the presence of calcium carbonate, fumaric acid accumulated in appreciable quantities, at times in yields representing 30 to 40 per cent of the sugar consumed. Succinic acid was present in the fermented solutions. When acetic acid was used as a carbon source, 80 per cent of the ether-soluble acid formed was succinic acid and 20 per cent fumaric acid, but when sugar was added to the acetic acid substrate the values for the respective acids were 26 and 74 per cent. Butkewitsch and Fedoroff are of the opinion that ethanol is an intermediate in the transformation of sugar to fumaric acid by molds.

Further work on the production of fumaric acid from various carbon sources by molds might possibly develop a fermentation whereby this compound could be produced economically. If used for the production of tartaric acid the yields of fumaric acid would have to be very high and the raw material quite inexpensive in order to compete with the well-established industry based on the recovery of tartrates from wine lees.

GALLIC ACID

Gallic acid was discovered by Scheele (*82*) prior to 1787 in studying the result of the action of a mold on a water infusion of gall nuts.

The raw material used in the production of gallic acid is the glucoside tannin. Before 1860 it was known that ferments were responsible for the formation of gallic acid, but it was thought that the reaction was brought about by enzymes in the plant material (*64*, *80*). In 1867 Von Tieghem (*96*) made a thorough study of the question and demonstrated that two groups of molds, *Aspergillus niger* and *Penicillium glaucum*, would produce gallic acid by their growth on tannin liquor and that air was necessary for the growth of both organisms. In this investigation, however, he reached the mistaken conclusion that the enzyme produced by the organisms would not operate except in the presence of the living plant.

Several years later two other investigators, Fernbach (*40*) and Pottevin (*78*), independently took up an investigation of the fermentation of tannin and reported that the enzyme tannase could be produced by the growth of *A. niger* on solutions containing tannin and suitable nutrients and could then be separated and used to hydrolyze solutions of pure tannin to gallic acid. The results of these two investigations prove conclusively that the hydrolysis of tannin to gallic acid is an extracellular reaction.

Knudson (*59*, *60*) found that the addition of tannic acid to Czapek's solution containing 10 per cent sugar causes a progressive increase in the tannase content of *A. niger* grown on it.

Gallic acid has long been produced industrially by the action of molds on tannin. The old process, described by Duclaux, (*31*) consisted of piling the moist tannin-containing material in heaps, maintaining them at temperatures of around 30° C., and stirring the heaps from time to time. The molds developed throughout the mass. After about a month the action was complete, and the gallic acid was leached out. The process has been much improved in the last 30 years, and most of the gallic acid made by the mycological process to-day is undoubtedly manufactured by the method described by Calmette (see patent) or some modification of it. Clear tannin extract is used, and pure cultures of one of the varieties of Aspergilli are generally employed. The sterile extract is inoculated and the solution agitated by blowing air through it and by mechanical agitators. Analysis of the mixture is made from time to time, and when the tannin has entirely disappeared the fermentation is terminated and the gallic acid recovered in the usual manner.

The mycological process has many advantages over the hydrolytic method often used and is being utilized industrially at present.

GLUCONIC ACID

The discovery of the microbiological production of gluconic acid was made by Boutroux (*12*), who in 1878 isolated an acid from culture solutions of *Mycoderma aceti* on glucose which he concluded was lactic, but which two years later he recognized as gluconic acid. It has since been frequently isolated from glucose solutions in which bacteria have been cultured (*47*, *p. 141–143*). In 1922 Molliard (*68*, *69*) separated the acid as the calcium salt, along with citric and oxalic acids, from cultures of *A. niger* on sucrose, and since then it has been recognized as a rather commonly occurring product of mold metabolism. The formation of gluconic acid apparently does not involve the complex series of reactions commonly found in most mold and bacterial fermentations. In a series of papers Müller (*71*, *72*) has described an enzyme preparation, which he obtained from press juices of Aspergilli and Penicillia, capable of transforming glucose to gluconic acid which he has termed "glucose-oxidase." Bernhauer (*10*) also obtained experimental results from which he concluded that the formation of gluconic acid was a strictly enzymic process induced by an oxidase which he termed "glucoxidase." He noted that toxic materials added to the fermenting solutions in concentrations sufficient to inhibit vegetative growth did not interfere with gluconic acid formation by a mycelium already developed, but that their addition completely suppressed citric acid formation, indicating that there is a wide fundamental difference in the mechanisms of the reactions leading to the formation of the two acids.

The ease with which certain fungi oxidize glucose to gluconic acid led May, Herrick, Moyer, and Hellbach (*65*) to investigate the pos-

sibilities of industrial production of the acid by the mold fermentation of solutions of commercial glucose. Using as containers pans fabricated from high grade aluminum, they were able to obtain yields of acid averaging around 57 per cent of the theoretical, from 20 per cent glucose solutions. The acid was recovered from the fermented solutions as the calcium salt, which, owing to its high solubility in hot water and relatively low solubility in cold water, is easily obtained in a high state of purity. It was demonstrated that this process has industrial possibilities, although it is possible that the acid might be produced more cheaply if chemical methods now in use for the oxidation of glucose were improved.

Recently Herman (*55*) has isolated a number of organisms from Kombucha, a fermented, sweetened tea. Among the bacteria present was a new type which fermented glucose quantitatively to gluconic acid and which he named *Bacterium gluconicum*. However, the fermentation induced by this organism is quite slow and apparently has little industrial significance, at least in its present state. On the other hand Takahashi and Asai (*89*) have found gluconic acid forming bacteria in Hoshigaki, the dried persimmon. This discovery seems to have industrial possibilities, as certain species of these bacteria have the ability to bring about a fairly rapid fermentation of glucose. Bacterial production of gluconic acid on an industrial scale is dependent largely on the isolation of new species of organisms capable of fermenting glucose at a rate comparable to that of the more active molds.

KOJIC ACID

The production of kojic acid (5-hydroxy-2-hydroxymethyl-1, 4-pyrone) from carbohydrates by Aspergilli of the oryzae-flavus group is an exceedingly interesting phenomenon. It was first observed by Saito (*81*), who, however, was unaware of the true nature of the compound which he isolated. Its constitution was established in 1924 by Yabuta (*117*), who spent some years investigating its chemical properties and its production by *Aspergillus oryzae*. Recently May, Moyer, Wells, and Herrick (*66*) have reviewed the literature to 1930 dealing with the formation of kojic acid by fungi. They investigated the ability of *A. flavus* to produce this compound from glucose and found that with 20 per cent solutions of this sugar the organism was capable of transforming more than 45 per cent of the dextrose in the substrate to kojic acid.

The mechanism by which sugars, such as sucrose, galactose, mannose, dextrose, arabinose, and xylose, and other compounds such as starch, inulin, glycerol, and gluconic acid, are transformed into kojic acid is quite obscure. The data accumulated thus far point to a synthesis from intermediates containing two or three carbon atoms. Recently Challenger, Klein, and Walker (*23*) have found that more than 30 per cent of the theoretical yield of kojic acid was obtained when *A. oryzae* was cultured on solutions containing dihydroxyacetone as the sole carbon source. This is strongly suggestive of a mechanism involving rupture of the sugar molecule, with the formation of a triose from which the kojic acid is then produced. Kojic acid, in quantities sufficient for experimentation, has not hitherto

been available in this country, and its utility in science and industry is therefore unknown. Should uses for it develop, its production from glucose by fermentation should offer no industrial difficulties.

LACTIC ACID

The formation of lactic acid by fermentation was first recognized as such by Blondeau (*11*) in 1847. Ten years later the lactic fermentation was investigated by Pasteur, and it served as a starting point for his contributions to the science of microbiology. Lister was the first to isolate pure cultures of lactic organisms (1877), and subsequently many different types of these bacteria were obtained in pure culture and named. Various systems for the classification of this group of bacteria have been suggested, but no single system has proved to be entirely satisfactory (*93*, *p. 133*).

With recognition of the importance of pure cultures and with consequent improvement of the fermentation, some attention was given to its industrial possibilities. The first successful production of lactic acid on a commercial scale was accomplished by Avery, an American, in 1881 (*48*). Production lagged somewhat, owing to lack of demand, but as industry grew in the United States and rule-of-thumb methods began to be displaced in various processes, the lactic acid fermentation was established on a sound commercial basis. The raw materials used were usually glucose or sucrose, and the yields of acid obtained were around 60 per cent of the theoretical amounts. At best the early methods were largely empirical, and a lactic fermentation entirely free from infection by butyric and alcoholic organisms was unusual. Many improvements have been made in recent years, mainly through the development of more successful methods of maintaining pure cultures, isolation of more productive strains, and better fermentation control. As practiced to-day, the process consists of a high-temperature (50° C.) fermentation of sterilized solutions of cane and beet sugar molasses, starch, or glucose, employing an organism of the Delbrucki type. When starch is used, it must first be hydrolyzed by either malt or acid. A sugar concentration of around 10 per cent is most generally used. Adjustment of the acidity of the raw mash to a pH of 3.7 to 4.5 permits an efficient sterilization by heating without employing excessive temperatures and pressures. The addition of nutrient salts is generally necessary. As the fermentation progresses, the accumulation of lactic acid affects the activity of the organisms, and it is therefore necessary to add a neutralizing agent. This is generally accomplished by adding the entire calculated quantity of calcium carbonate at the beginning of the fermentation, although it may be added intermittently as the formation of acid takes place. The latter procedure is desirable, since the maintenance of a slight acidity in the mash minimizes the danger of infecting organisms obtaining a foothold, but it necessitates rigid control of the fermentation if maximum yields are to be obtained. The sugar, as a rule, completely disappears in from four to six days. The calcium salt is recovered, and the lactic acid liberated with sulphuric acid. The yield in a well-controlled process is around 90 per cent of the theoretical.

The lactic fermentation has been intensively investigated from the theoretical viewpoint with the object of clarifying the mechanism of the reactions involved. No attempt will be made to review the literature (*47, p. 145–151; 87, p. 50–65*). Present knowledge indicates that the fermentation is initiated with the formation of hexose phosphates, which break down into a 3-carbon compound, probably methyl glyoxal, which, through the action of the enzyme glyoxalase, is transformed to lactic acid.

MALIC ACID

Malic acid has from time to time been reported as occurring in rather small quantities among the products of yeast fermentations. Dakin (*30*) has made an investigation of the formation of the levo acid in the alcoholic fermentation of sugars by yeast. He reported that the addition of sodium bicarbonate and especially sodium fumarate had a very marked effect in increasing the quantity of malic acid formed. Kostytschew and Frey (*61*) have reported the formation of this acid in appreciable quantities in rapid alcoholic fermentations brought about in the presence of calcium carbonate and in the absence of nitrogenous nutrients. They are of the opinion that it probably is derived from hydroxyglutamic, aspartic, or succinic acids resulting from the degradation of yeast protein. Wehmer (*111*) has reported malic acid among the final products resulting from the action of *Aspergillus fumaricus* on sucrose, but in a later investigation with the same organism, Thies (*94*) was unable to detect its presence. The origin of malic acid in fermentations is, at the present, quite obscure, but its close structural relationship to succinic and fumaric acids suggests a common precursor.

OXALIC ACID

The accumulation of oxalic acid in carbohydrate cultures of molds has been observed with varieties of Aspergilli, Penicillia, Mucores, and other less common organisms. Among the earliest recorded observations is that of De Bary (*4*) who reported the formation of soluble oxalates in cultures of *Sclerotinia sclerotiorum*. Shortly thereafter Hansen (*53*) observed the appearance of oxalic acid in gelatine cultures of so-called *Penicillium glaucum*, and Duclaux (*32*) reported its formation in carbohydrate cultures of *Aspergillus niger*. In 1891 Wehmer (*102*) reported the production of large quantities of this acid from sucrose by a strain of *A. niger* and subsequently carried out a systematic study of conditions affecting its occurrence (*102, 103, 104, 107*). Under definite cultural conditions 10 grams of oxalic acid was produced from 15 grams of sucrose. The early literature of the biochemical production of oxalic acid by bacteria and molds has been reviewed by Banning (*3*). Currie and Thom (*29*) studied the ability of Penicillia and Aspergilli to produce oxalic acid, and incidentally this survey led to the discovery of the production of citric acid by *A. niger*.

The work of the last 15 years has indicated that the accumulation of oxalic acid in mold cultures is dependent largely on experimental factors and that in citric or gluconic fermentations this accumulation can be almost completely eliminated by a proper choice of the

conditions under which the process is carried out. In a recent paper dealing with the occurrence of oxalic acid in mold cultures, Chrzaszcz and Tiukow (*26*, *27*) have reviewed the literature thoroughly. They concluded that the presence of amino substances in the substrate influenced favorably the accumulation of oxalic acid which was formed at the expense of the sugar.

Despite the ability of many organisms of the Aspergillus and Penicillium species to produce large yields of oxalic acid from various carbohydrates, this fermentation has no commercial significance, owing to the ease of manufacturing the acid by strictly chemical methods.

PROPIONIC ACID

In 1841 Nöllner (*75*) isolated a substance from the products of spontaneous fermentation of moist calcium tartrate which he designated pseudo-acetic acid. Study of the properties of some of the salts of this acid indicates that he was dealing, at least in part, with propionic acid. The same acid was reported by different investigators under different names, such as "metacetonic acid" and "butyro-acetic" acid. Dumas, Malagutti and Leblanc (*33*) established that the three acids were identical and proposed the name "propionic acid" for the compound.

Early reports of the occurrence of propionic acid among fermentation products are not of great value, as in no case was the causative organism isolated from or apparently even predominant in the fermenting material.

While investigating the formation of "eyes" in Swiss cheese, Jensen (*57*) found a relation between the number and size of the eyes and the quantity of propionic acid in the cheese. Shortly after, Von Freudenreich and Jensen (*46*) succeeded in isolating propionic acid bacteria which they designated *Bacteria acidi propionici.* These bacteria occur widely and in fairly large numbers in dairy products, such as milk, cheese, and rennet.

While trying to determine the reason for the inferiority of American cheeses of the Emmenthaler type as compared with the European product, Sherman and Shaw (*86*) made a detailed study of the propionic organisms in that material. As a result of this work, vigorous strains of *Bact. acidi propionici* were isolated, having such strong propionic acid producing characteristics as to inspire a study of the possibility of large-scale production of the acid by fermentation (*113*, *114*). It was found that carbohydrates, in particular the lactose of sterilized whey, could be fermented to propionic and acetic acids, the action being complete in 10 days when carried out at 38° C. An interesting feature brought out in this investigation was that the rate of acid production and the ratio of propionic to acetic acid were greatly increased by the presence of other organisms, such as *Streptococcus lactis*, *Lactobacillus casei*, and *Proteus vulgaris*, in the mash. This phenomenon has been confirmed by Van Niel, who ascribed the stimulating effect to an alteration of the nitrogenous compounds of the medium. Recently Van Niel has completed a thorough review of the whole question of bacterial production of propionic acid (*74*). He made detailed studies of the various organisms, suitability of different carbon sources, variations

in nutrient media, and relations of the bacteria to oxygen. The interested reader should consult this treatise for specific information on almost any phase of propionic acid fermentation.

Foote, Fred, and Peterson (*43*) have recently investigated the fermentation of pentoses by 20 different cultures of propionic acid bacteria, of which 13 were able to ferment arabinose and one to ferment xylose. These sugars differed from glucose mainly in that there was a decrease in the percentage of volatile acids and a corresponding increase in nonvolatile acids. Werkman and his associates (*112*) have noted the ability of *Propionibacterium pentosaceum* to attack xylose and arabinose with the production of propionic and acetic acids.

Virtanen (*98*, *99*) has carried out an important series of experiments with a view to formulating a mechanism which might express the reactions involved in this fermentation. The organism used was isolated from cheese and was capable of converting glucose, and pyruvic and lactic acids to propionic and acetic acids in the ratio of 2 to 1. Experiments with glucose indicated that it is esterified with phosphoric acid as an initial step in the fermentation. As in the lactic fermentation, the organism possesses a cozymase complex, which may be removed by washing. An interesting fact is that the phosphatase coenzyme of lactic acid will reactivate the dried and washed propionic bacteria as will also the wash water of the dried bacteria themselves. Virtanen also observed in his experiments the formation of succinic acid, which he claims arises from the sugar, in quantities up to 25 per cent of the sugar fermented. This unusually large production of succinic acid has been questioned, but Foote, Peterson, and Fred (*43*) found such quantities of the acid in the propionic fermentation of pentoses that they concluded that it must be derived from the sugar. The work of Neuberg and Gorr (*73*) indicated that the propionic acid bacteria contain ketone-aldehyde mutase, since the dried organisms easily convert methyl glyoxal to lactic acid. On the basis of results so far obtained, Virtanen suggests the following scheme for representing the changes occurring in the propionic fermentation induced by the organisms which he investigated:

Glucose→hexose phosphate→lactic acid→propionic acid; succinic
↓
acid and acetaldehyde→acetic acid and carbon dioxide. The establishment of the mechanism of this fermentation, however, can not in any manner be considered final. Van Niel (*74*, *p. 38*) has stated the problem very well, as follows:

> The peculiar phenomenon of the stimulating effect of foreign microbes on the propionic acid fermentation is wholly lacking in explanation, while the experiments of Virtanen and of Neuberg and Gorr are still in want of important additional data before any clear insight in the chemism may be attained.

Whittier and Sherman (*113*) have called attention to the utility of propionic acid esters as solvents for cellulose derivatives and have also pointed out the advantages of propionic over acetic acid as a solvent in some cases, owing to the wide range of temperatures over which it remains liquid. The possibility of preparing ketones from calcium propionate has also been suggested. However, the length of time required for the fermentation (10 days) and the

necessarily low concentration of acid in the final fermented material (5 per cent solutions of lactose were found to be best) have apparently discouraged any industrial application of the work of Sherman and his associates. Nevertheless, Van Niel (*74, p. 181–187*), as a result of his experimental work, is of the opinion that the propionic fermentation has distinct industrial possibilities. He found that the difficulties caused by the time required for a complete fermentation could be overcome by carrying out the process on a semicontinuous basis. Thus, complete fermentation of the substrate required from 7 to 10 days. After replacement of the mash five times, the final batch was completely fermented in two days. The ratio of propionic acid to acetic acid can be varied as desired by proper selection of organism and nutrient nitrogen and, although the ratio is two to one under usual conditions, certain strains will produce the acids from glucose in a ratio of five to one. The choice of organism is also influenced by the raw material available for the fermentation, as some strains work best on lactose, whereas others are most efficient on starch or dextrin. Thus, Wilson, Fred, and Peterson (*115*) have studied the comparative action of 11 strains of propionic acid bacteria on solutions of glucose, sucrose, maltose, and lactose. With *Lactobacillus casei*, glucose gave the best yields. Sucrose and lactose followed closely, but maltose gave variable results. Selected strains vigorously fermented molasses and hydrolyzed starch solutions. Thus, 80 per cent of the carbohydrate of the latter (3.8 grams glucose per 100 cubic centimeters solution) was fermented in from four to five days. Over a 14-day period, 93 to 95 per cent of the sugar disappeared, and from 45 to 50 per cent of this was accounted for as volatile acids, which consisted of 65 to 70 per cent propionic acid.

From a consideration of the present state of the propionic fermentation, it appears that the production of propionic acid by this method might prove feasible. Establishment of the process on an industrial scale would entail considerable research and once established would require rigid control for successful operation. As demand for the acid developed, competition would undoubtedly arise from the synthetic chemical industry, and the final success of the fermentation process would depend on its efficiency, the price of raw material, the cost of recovery of the desired products, and the volume of production.

SUCCINIC ACID

Pasteur (*76*), in his paper on alcoholic fermentation, first reported the formation of succinic acid by microorganisms. The acid has since been isolated in small quantities from the final products of a wide variety of yeast, bacterial, and mold fermentations. The work of Ehrlich (*34*), Kostytschew and Frey (*61*), and others demonstrated that in a large number of fermentations succinic acid is formed from the glutamic acid which arises from the decomposition of protein material. On the other hand, it is certain that in some cases succinic acid is formed from the sugar of the substrate. Thus the large quantities of succinic acid found in his propionic acid fermentation led Virtanen (*100*) to the belief that it is formed by the propionic organisms directly from the hexose without intervention of coenzymes, as is indicated in the section of this circular dealing

with propionic acid. Foote, Fred, and Peterson (*43*), in their investigation of the propionic fermentation of pentoses, observed the formation of comparatively large quantities of succinic acid (as much as 20 grams of the acid for each 100 grams of arabinose fermented) and concluded that it was derived from the carbohydrate rather than from the yeast water used in the substrate. Butkewitsch and Fedoroff (*22*), as has been stated elsewhere, have noted the formation of appreciable quantities of succinic acid from sugars, ethanol, and acetic acid by a strain of *Mucor stolonifer*, and they have suggested that the succinic acid is derived from acetic acid, which in turn is formed either by oxidation of ethanol or by dismutation of acetaldehyde. This mechanism, according to them, might apply equally well to the formation of the acid in the alcoholic fermentation induced by yeasts.

Recent work thus points to the possibility that a fermentation of sugar to succinic acid in which the yields of the acid might have an economic significance may eventually be found.

PATENT LITERATURE

ACETIC ACID

(1) Backhaus, A. A., and Haner, C., Jr.
METHOD OF TREATING DISTILLERY WASTE. U. S. Pats. 1,396,006; 1,396,007, November 8, 1921; appl. October 22, 1917.
Distillery slop is treated with a solution of sodium hydroxide, the mixture is dried to a clinker, and the acids are recovered by treatment with acid.

(2) Basset, H. P.
PROCESS OF TREATING DISTILLER'S SLOP. U. S. Pat. 1,344,851, June 29, 1920; appl. July 12, 1919.
Distillery slop is treated with lime water under pressure for the separation and recovery of acetic and formic acids.

(3) Bernier, M., Duriez, A., Duriez, F., and Schotsmans, H.
INDUSTRIAL PROCESS FOR THE TREATMENT OF DISTILLER'S WASH AND OTHER BY-PRODUCTS AND AGRICULTURAL OR INDUSTRIAL RESIDUES. U. S. Pat. 1,747,538, February 18, 1930; appl. March 29, 1923.
Wastes, such as distiller's wash, are sterilized, neutralized, and fermented with *Bacillus aminophilus*.

(4) Craney, T.
IMPROVEMENTS IN APPARATUS FOR THE PRODUCTION OF VALUABLE PRODUCTS FROM REFUSE ORGANIC MATTER. Brit. Pat. 4,922, October 21, 1915; appl. March 30, 1915.
Refuse material, such as garbage, is allowed to ferment with or without the addition of suitable organisms, and acetic acid is recovered by means of special equipment.

(5) Distillers Company, Limited, and Hutchinson, H. B.
IMPROVEMENTS IN OR RELATING TO THE PRODUCTION OF ALIPHATIC ACIDS BY FERMENTATION. Brit. Pat. 337,153, October 30, 1930; appl. September 30, 1929.
Solutions containing carbohydrates, including molasses, are fermented by thermophilic organisms obtained from animal excrement or decomposing cellulosic material, to acetic, butyric, and propionic acids. The fermentation is controlled so as to repress the formation of nonvolatile acids.

(6) Langwell, H.
IMPROVEMENTS IN OR RELATING TO PROCESSES FOR FERMENTING CELLULOSIC MATERIALS. Brit. Pat. 248,795, March 10, 1926; appl. September 10, 1924. U. S. Pat. 1,602,306, October 5, 1926; appl. September 12, 1925.
Improvement in cellulosic fermentation is obtained by maintaining the hydrogen-ion concentration between 10^{-5} and 10^{-9} and the temperature between 60° and 70° C.

(7) Norbury, H. R., Desborough, A. P. H., Thaysen, A. C., and Galloway, L. D.

IMPROVED PROCESS FOR TREATING VEGETABLE MATTER BY FERMENTATION TO OBTAIN ALCOHOL, ACETONE AND OTHER PRODUCTS. Brit. Pat. 222,549, September 29, 1924; appl. June 27, 1923.

Hydrolyzed straw and grasses are fermented with *Bacillus acetoethylicus* to produce ethanol, acetone, and acetic acid.

(8) Power-Gas Corporation, Limited, and Langwell, H.

IMPROVEMENTS IN THE FERMENTATION OF CELLULOSE. Brit. Pat. 134,265, November 3, 1919; appl. October 1, 1918; U. S. Pat. 1,443,881, January 30, 1923, appl. September 29, 1919.

Fermentation of cellulosic material for the production of acetic acid is carried out by conducting the operation aerobically with organisms introduced by adding fermenting vegetable matter.

(9) ——— and Langwell, H.

IMPROVEMENTS IN THE FERMENTATION OF CELLULOSE. Brit. Pat. 161,294, April 8, 1921; appl. January 8, 1920.

Improvement in the process outlined in Brit. Pat. 134,265 and U. S. Pat. 1,443,881 is obtained by regulating the degree of aeration with the object of influencing the type of products produced.

See also lactic and butyric acid patents.

BUTYRIC ACID

(1) Desparmet, E.

PROCÉDÉ DE PRODUCTION DE L'ACIDE BUTYRIQUE ET DES ACIDES DE LA SÉRIE GRASSE PAR FERMENTATION DIRECTE DES FARINES, AMIDONS, ET DES PRODUITS AMYLACÉS SANS SACCHARIFICATION PRÉALABLE. Fr. Pat. 662,321, March 18, 1929; appl. February 10, 1928.

Amylaceous substances are fermented by pure or symbiotic cultures of butyric bacteria.

(2) Dupont, L.

PROCESS FOR THE UTILIZATION OF MARINE ALGAE FOR THE MANUFACTURE OF ACETIC AND BUTYRIC ACIDS. U. S. Pat. 1,371,611, March 15, 1921; appl. March 6, 1919.

Fresh algae are spontaneously fermented with earth fertilized with sea wrack.

(3) Effront, J.

PROCÉDÉ DE RÉCUPERATION D'AMMONIAQUE PAR LE FERMENTATION DES SUBSTANCES ORGANIQUES AZOTÉES. Fr. Pat. 382,689, December 14, 1907; appl. October 8, 1907. Brit. Pat. 9,951, November 26, 1908; appl. May 7, 1908. U. S. Pat. 953.025, March 29, 1910; appl. May. 14, 1908.

Waste products from alcohol distilleries or sugar factories, sewage, turf, etc., are fermented to acetic, propionic, and butyric acids. The acids arise from protein material and amino acids present in the mash.

(4) Hibbert, H.

SOLVENT AND PROCESS OF PRODUCING THE SAME. U. S. Pat. 1,283,183, October 29, 1918; appl. October 27, 1915, renewed March 19, 1918.

Suitable carbohydrate solutions are inoculated with lactic bacteria, and the lactic acid produced is fermented to butyric acid by butyric organisms or the carbohydrate mash may be directly fermented to butyric acid. The acids produced are transformed into ketones by subsequent chemical treatment.

(5) Lefranc, L.

MANUFACTURE OF BUTYRIC ACID AND OTHER ALIPHATIC ACIDS. U. S. Pat. 1,625,732, April 19, 1927; appl. August 18, 1922.

Fermentation of hydrolyzed cellulosic material is carried out at 40° C. by bacteria obtained from the intestines of herbivora.

(6) McDermott, F. A., and Glasgow, R.

MANUFACTURE OF BUTYRIC ACID. U. S. Pat. 1,405,055, January 31, 1922; appl. December 13, 1917.

Slops from alcoholic fermentation are fermented, and butyric acid is recovered as copper butyrate.

(7) Sinclair, W. M.

PROCESS OF MANUFACTURE OF ALDEHYDES, OILS, AND ORGANIC ACIDS FROM CACTI. U. S. Pat. 1,569,339, January 12, 1926; appl. February 25, 1925.

Fermentation of crushed pricklypear plants is carried out by means of organisms naturally present on the plant.

(8) Société Darrasse Frères, and Dupont, L.

PROCÉDÉ D'UTILISATION DES ALGUES MARINES POUR LA FABRICATION DES ACIDES FORMIQUE, ACETIQUE, BUTYRIQUE ET L'EXTRACTION DES SELS D'IODE ET DE POTASSE. Fr. Pat. 520,456, February 15, 1921; appl. February 13, 1918. Brit. Pat. 123,325, May 13, 1920; appl. February 13, 1919.

Same as U. S. Pat. 1, 371, 611 (Reference 2).

(9) ——— and Dupont, L.

PROCÉDÉ INDUSTRIELLE D'UTILISATION DES MATIÈRES HYDROCARBONÉES CONTENUES DANS LES VÉGÉTAUX, LES DÉBRIS VÉGÉTAUX ET LES DÉCHETS NATURELS OU INDUSTRIELS. Fr. Pat. 520,457, February 15, 1921; appl. February 13, 1918. Brit. Pat. 123,326, May 13, 1920; appl. February 13, 1919.

Carbohydrates in waste products, such as bisulphite pulp liquors, tannin waste, and grass, are fermented to formic, acetic, and butyric acids.

(10) Société d'Étude de Carburx.

PROCÉDÉ DE FABRICATION INDUSTRIELLE DES ACIDES DE LA SÉRIE GRASSE ET EN PARTICULIER DE L'ACIDE BUTYRIQUE. Fr. Pat. 469,552, May 23, 1914; appl. March 9, 1914.

Cellulose, starch, and sugars are fermented by a species of Clostridium at 38° C. in the presence of neutralizing agents.

(11) Société Lefranc & Cie.

PROCÉDÉ DE PRODUCTION DE L'ACIDE BUTYRIQUE ET DES ACIDES DE LA SÉRIE GRASSE. Fr. Pat. 541,535, May 4, 1922; appl. September 26, 1921.

Hydrolyzed cellulosic material is fermented by butyric bacteria alone or in symbiosis with putrefactive organisms, such as *Bacillus subtilis* and *Bacillus albuminus*. Addition No. 29,404 (1924) states that by selective culture organisms may be obtained which give higher yields of fatty acids, chiefly butyric, at temperatures of 42° to 44° C.

CITRIC ACID

(1) Bleyer, B.

VERFAHREN ZUR HERSTELLUNG VON CITRONENSÄURE. Ger. Pat. 434, 729, October 6, 1926; appl. October 19, 1924.

Carbohydrate solutions to which acid has been added to bring the pH to 3.3–3.6 are fermented by Aspergilli, Citromyces, or Penicillia. Sterile air is passed over the fermentation pans to remove excess CO_2.

(2) Cahn, F. J.

PERFECTIONNEMENTS À LA FABRICATION DE L'ACIDE CITRIQUE PAR FERMENTATION. Fr. Pats. 675,236, and 675,237, October 29, 1929; appl. May 16, 1929.

Sections of sugar-containing plants or plant residues impregnated with sugar solutions are fermented by Aspergilli or other citric acid producing fungi.

(3) The Distillers Company Limited, and Peake, A. M.

IMPROVEMENTS RELATING TO THE PRODUCTION OF CITRIC ACID. Brit. Pat. 287,604, March 27, 1928; appl. November 27, 1926. U. S. Pat. 1,788,739, January 13, 1931; appl. November 25, 1927.

Pectinous substances, dextrins, etc., are converted to citric acid by enzymes contained in the pith of citrus fruits.

(4) Fabriques de Produits Chimiques de Thann et de Mulhouse.

DARSTELLUNG VON CITRONENSÄURE DURCH GÄHRUNG. Ger. Pat. 91,891, April 13, 1897; appl. April 5, 1893.

Carbohydrates are fermented to citric acid by *Mucor piriformis*.

(5) Falck, R.

VERFAHREN ZUR HERSTELLUNG VON CITRONENSÄURE UND ANDEREN ORGANISCHEN SÄUEREN. Ger. Pat. 426,926, March 29, 1926; appl. May 31, 1921.

Solid, starchy substrates are fermented by fungi of the Aspergillus, Penicillium, or Citromyces groups.

(6) Fernbach, A., and Yuill, J. L.

IMPROVEMENTS IN AND RELATING TO PROCESSES FOR THE PRODUCTION OF CITRIC ACID. Brit. Pats. 266,414, 266,415, February 28, 1927; appl. October 26, 1925. Fr. Pats. 610,121, 610,122, May 29, 1926; appl. January 21, 1926. U. S. Pats. 1,691,965, 1,691,966, November 20, 1928; appl. November 3, 1925.

Carbohydrate solutions, effectively sterilized by the addition of acids to bring the pH to 1.2–2.5, are fermented to citric acid by dark-colored Aspergilli.

(7) KANHÄUSER, F.

PROCESS FOR THE PRODUCTION OF CITRIC ACID BY FERMENTATION. U. S. Pat. 1,779,001, October 21, 1930; appl. August 31, 1928.

Improvements in the production of citric acid by mold fermentation are obtained by cultivating the organisms in alternating stages on solid agar media, on media corresponding to those used industrially, and on vegetable media containing organic acids and vitamins. The presence in the mash of substances which, with citric acid, form complexes of high molecular weight, is claimed to be beneficial.

(8) MONTAN- UND INDUSTRIALWERKE VORMALS JOH. DAV. STARCK.

VERFAHREN ZUR HERSTELLUNG VON GÄRUNGSZITRONENSÄURE. Ger. Pat. 461,356, June 13, 1928; appl. October 5, 1924.

Molasses solutions are fermented at temperatures of from 15° to 30° C. by citric acid producing fungi.

(9) SCHOLVIEN, K.

VERFAHREN ZUR HERSTELLUNG GEGORENER ALKOHOLFREIER GETRÄNKE MIT BIERARTIGEN AROMA AUS BIERWÜRZE. Ger. Pat. 162,622, September 13, 1905; appl. August 14, 1904.

A nonalcoholic beverage is prepared by inoculating hopped or unhopped beer wort with a citric acid forming organism such as Citromyces.

(10) SZÜCS, J.

VERFAHREN ZUR HERSTELLUNG VON GÄRUNGSZITRONENSÄURE. Austrian Pat. 101,009, September 25, 1925; appl. December 19, 1923. Canadian Pat. 251,180, June 30, 1925; appl. December 4, 1924. U. S. Pat. 1,679,186, July 31, 1928; appl. December 4, 1924.

Same as Ger. Pat. 461,356 (Reference 8).

(11) WEHMER, C.

DARSTELLUNG VON CITRONENSÄURE DURCH GÄHRUNG. Ger. Pat. 72,957, February 20, 1894; appl. April 5, 1893. Brit. Pat. 5,620, December 9, 1893; appl. March 15, 1893. Fr. Pat. 228,554, March 11, 1893. U. S. Pat. 515,033, February 20, 1894; appl. April 3, 1893.

Citric acid is produced from carbohydrates by the action of citric acid forming fungi.

(12) ZAHORSKI, B.

METHOD OF PRODUCING CITRIC ACID. U. S. Pat. 1,066,358, July 1, 1913; appl. January 26, 1910.

Sugar is converted into citric acid by the action of *Sterigmatocystis nigra.*

FUMARIC ACID

(1) WEHMER, C.

IMPROVED MANUFACTURE OF FUMARIC ACID. Brit. Pat. 146,411, October 3, 1921; appl. July 2, 1920.

Carbohydrates are fermented to fumaric acid by pure cultures of *Aspergillus fumaricus.*

GALLIC ACID

(1) CALMETTE, A.

VERFAHREN ZUR UMWANDLUNG VON TANNIN IN GALLUSSÄURE. Ger. Pat. 129,164, March 1, 1902; appl. August 12, 1900.

Tannin-containing solutions are hydrolyzed by molds isolated from tannin-bearing materials.

GLUCONIC ACID

(1) BERNHAUER, K.

PROCÉDÉ D'OBTENTION INDUSTRIELLE DE L'ACIDE GLUCONIQUE PAR VOIE DE FERMENTATION. Fr. Pat. 707,614, July 10, 1931; appl. December 16, 1930.

The glucose of sucrose, molasses, chopped sugar beets, hydrolyzed starch, etc., is oxidized to gluconic acid in the presence of calcium carbonate by molds such as Aspergillus, Penicillium, Citromyces or Mucor, the mycelia being lifted at intervals to permit the escape of carbon dioxide. Various substances are added to the mash to repress the activities of undesirable organisms.

(2) HERMANN, S., UND PHARMACEUTISCHE WERKE "NORGINE" A. G. IN PRAG.
VERFAHREN ZUR HERSTELLUNG VON GLUCONSÄURE AUS KOHLEHYDRATEN AUF BIOLOGISCHEM WEGE. Ger. Pat. 522,147, April 1, 1931; appl. February 20, 1927.
Glucose or sucrose solutions are oxidized to gluconic acid by bacteria in the sweetened Japanese tea known as Kombucha. Acetic acid is also formed during the fermentation.

(3) HERRICK, H. T., and MAY, O. E.
PROCESS FOR THE MANUFACTURE OF GLUCONIC ACID. U. S. Pat. 1,726,067, August 27, 1929; appl. July 28, 1927.
Gluconic acid is produced from glucose by the action of fungi from the groups *Penicillium citrinum, P. divaricatum* and *P. luteum purpurogenum.*

LACTIC ACID

(1) AVERY, C. E.
MANUFACTURE OF LACTATES. U. S. Pat. 243,827, July 5, 1881; appl. December 21, 1880. Fr. Pat. 143,979, July 16, 1881.
Sugar solutions containing neutralizing agents are fermented in the presence of nitrogenous matter at a temperature of 35° to 40° C.

(2) ———
MANUFACTURE OF LACTATES. U. S. Pat. 330,815, November 17, 1885; appl. November 2, 1883.
Pure lactic ferments are prepared on a large scale by carrying out a series of successive inoculations on media unfavorable for the growth of contaminating organisms.

(3) BAKONYI, S. (DEUTSCHE HYDRIERWERKE AKTIENGESELLSCHAFT, ASSIGNEE.)
AN IMPROVED METHOD OF CARRYING OUT BIOCHEMICAL PROCESSES. Brit. Pat. 293,015, July 15, 1929; appl. April 13, 1928.
A method is described for the isolation of organisms having a resistance to lactic acid greater than that of the usual strains.

(4) BECKERS, W.
VERFAHREN ZUR GEWINNUNG VON MILCHSÄURE AUS ABWASSERN. Ger. Pat. 113,383, Aug. 10, 1900; appl. September 18, 1898.
Lactic acid from waste wash waters resulting from the manufacture of sauerkraut is separated and recovered.

(5) BLANS, K.
VERFAHREN ZUR TRENNUNG DER IN' DEN BEI DER REISVERARBEITUNG ENTSTEHENDEN ABFALLPRODUKTEN ENTHALTENEN VERWERTHAREN BESTANDTEILE. Swiss Pat. 49,532, September 8, 1909.
By-product waste from the manufacture of rice meal is treated with diastatic enzymes, and the filtrate is fermented to lactic and acetic acids. The residue is treated for the recovery of fats and proteins.

(6) BONELLI, A., and GULMINELLI, G.
PROCÉDÉ POUR LA FABRICATION INDUSTRIELLE DE L'ACIDE LACTIQUE DU COMMERCE. Fr. Pat. 490,589, January 4, 1919; appl. June 21, 1918.
Beets and molasses are fermented to lactic acid, and the acid is recovered in a special manner.

(7) BYK-GULDENWERKE CHEMISCHE FABRIK AKT.-GES.
VERFAHREN ZUR GEWINNUNG VON MILCHSÄURE. Ger. Pat. 358,110, September 5, 1922; appl. December 18, 1919.
Spoiled and rotted potatoes are used as raw material for the production of lactic acid by fermentation.

(8) CLAFLIN, A. A.
PROCESS OF MANUFACTURING LACTATES. U. S. Pat. 861,163, July 23, 1907; appl. June 14, 1907.
Lactic fermentation of suitable worts is carried out in the presence of neutralizing agents with the addition of fermentable materials as the fermentation proceeds.

(9) CUISINIER, M.
PROCÉDÉ DE FABRICATION DES LACTATES ET DE L'ACIDE LACTIQUE PAR FERMENTATION ET APPLICATIONS INDUSTRIELLES DIVERSES DE CES PRODUITS À LA FABRICATION D'ALCOOL, ETC. Fr. Pat. 181,774, July 24, 1887.
Lactic fermentations of infusions of grains treated with malt are carried out in the presence of carbonates at a temperature of 45 C°.

(10) DESBOROUGH, A. P. H., REILLY, J., and THAYSEN, A. C.
IMPROVEMENTS IN OR RELATING TO THE MANUFACTURE OF LACTIC ACID AND ITS SALTS AND THE RECOVERY OF THE VOLATILE FATTY ACID BY-PRODUCTS. Brit. Pat. 128,687, July 3, 1919; appl. April 20, 1918.
Sugars are fermented with pure cultures of *Bacterium volutans*.

(11) FAITHFULL, S. E.
MANUFACTURE OF LACTIC ACID. U. S. Pat. 1,569,221, January 12, 1926; appl. May 8, 1924. Fr. Pat. 607,995, April 10, 1926; appl. December 15, 1925. Brit. Pat. 265,336, February 10, 1927; appl. December 8, 1925.
The formation of undesirable by-products, such as butyric acid, in the lactic fermentation is prevented by aerating the fermenting solutions.

(12) FRIEDBERGER, O.
VERFAHREN ZUR HERSTELLUNG VON GÄRUNGSMILCHSÄURE AUS DEXTROSE. Ger. Pat. 258,050, March 19, 1913; appl. February 19, 1911. Brit. Pat. 2,507 (A. D. 1911), January 18, 1912; appl. January 31, 1911. Brit. Pat. 17,275 (A. D. 1911), January 31, 1912; appl. January 31, 1911. U. S. Pat. 1,074,708, October 7, 1913; appl. January 18, 1912.
Sugars are fermented to lactic acid by a strain of *Bacillus delbrücki* which has been accustomed to dextrose. The fermentation may be completed by adding cultures of *Bacterium acidi lactici* and *Bacterium bulgaricus*.

(13) FRED, E. B., and PETERSON, W. H.
FERMENTATION OF SOLUTIONS CONTAINING XYLOSE, ARABINOSE, AND OTHER PENTOSE SUGARS WITH THE PRODUCTION OF ACETIC AND LACTIC AND OTHER ORGANIC ACIDS BY MICROORGANISMS. U. S. Pat. 1,485,844, March 4, 1924; appl. August 7, 1919.
Pentose-containing hydrolysates of corn cobs, cottonseed hulls, etc., are fermented to acetic and lactic acids by cultures of *Lactobacillus pentoaceticus*.

(14) GARTH, H., and OSTLER, J.
EXTRACTING ALCOHOL, ACETIC ACID, ETC., FROM LOCUST BEANS. Brit. Pat. 3,331 (A. D. 1877), February 26, 1878; appl. September 1, 1877.
Locust meal or beans are hydrolyzed with dilute acid and extracted with water, and the extract is fermented either to alcohol or to lactic and butyric acids.

(15) HAMBURGER, T.
PURIFYING LACTIC ACID. U. S. Pat. 1,459,395, June 19, 1923; appl. September 23, 1921.
Lactic fermentations are carried out with neutralization of the excess acid with a suitable magnesium salt.

(16) JUST, J. A.
PROCESS OF MAKING LACTIC ACID. U. S. Pat. 868,444, October 15, 1907; appl. November 21, 1906.
Crude milk mixed with silica, asbestos, etc., is dried and leached with water. Calcium carbonate is added, and the extract is fermented at 27° to 40° C. by lactic organisms contained in rennet.

(17) LINDER, P.
VERFAHREN ZUR GLEICHZEITIGEN GEWINNUNG VON KOHLENSÄURE, ALKOHOL UND MILCHSÄURE. Ger. Pat. 478,558, June 28, 1929; appl. August 17, 1927. Brit. Pat. 295,648, December 6, 1928; appl. July 10, 1928.
Sugars are fermented to ethanol and lactic acid by bacteria isolated from the American aloe.

(18) LORENTZ, L. VON
AN IMPROVED PROCESS FOR RAISING PERMANENT PURE CULTURES OF LACTIC ACID BACTERIA. Brit. Pat. 7,898 (A. D. 1896), January 9, 1897, appl. April 14, 1896.
A method is described for the preparation of cultures of lactic bacteria in a form whereby they retain their vitality.

(19) LUTHER, M. and BELLER, H.
VERFAHREN ZUR GEWINNUNG VON MILCHSÄURE UND IHREN DERIVATEN. Ger. Pat. 472,914, March 14, 1929; appl. February 13, 1927. Brit. Pat. 290,464, May 17, 1928; appl. July 2, 1927. U. S. Pat. 1,766,715, June 24, 1930; appl. February 4, 1928.
Crude materials, such as molasses or potatoes, are fermented to lactic acid in the presence of calcium carbonate, the resulting mixture is dried, and the calcium lactate is extracted with methanol.

(20) MARSH, G. A.

MANUFACTURE OF LACTATES AND LACTIC ACID. U. S. Pat. 290,252, December 18, 1883; appl. September 26, 1883.

Gums and dextrin are fermented in the presence of neutralizing agents at a temperature of 40° C. to 45° C. without agitation of the fermenting solution.

(21) MISLIN, E. A., and LEWIN, L.

PROCEDE DE FABRICATION DE L'ACIDE LACTIQUE. Fr. Pat. 355,520, September 2, 1905; appl. May 16, 1905. Brit. Pat. 10,436 (A. D. 1905), May 3, 1906; appl. May 18, 1905.

Malted barley and corn are fermented with pure cultures of lactic bacteria with intermittent neutralization of the mash as the fermentation progresses.

(22) NOWAK, O. H.

SUBSTANCE FOR TREATING HIDES AND PROCESS OF MAKING THE SAME. Brit Pat. 26,771 (A. D. 1905), December 20, 1906; appl. December 22, 1905.

Skimmed milks, after removal of the curd, are fermented chiefly to lactic acid by the addition of sour rye dough. The fermented liquors are used directly in the treatment of hides, etc.

(23) NOWELL, T. S.

MANUFACTURE OF LACTIC ACID AND THE LACTATES, ETC. Brit. Pat. 5,780 (A. D. 1883), June 14, 1884; appl. December 18, 1883.

Starch is fermented to lactic acid in the presence of neutralizing agents.

(24) ———

IMPROVEMENTS IN THE MANUFACTURE OF LACTIC ACID AND LACTATES. Brit. Pat. 14,001 (A. D. 1885), August 16, 1886; appl. November 17, 1885. Fr. Pat. 172,345, November 17, 1885.

Lactic fermentations are carried out by organisms almost entirely free from butyric, alcoholic, and putrefactive ferments, and obtained in a special manner.

(25) PETERSON, W. H., and FRED, E. B.

PRODUCTION OF LACTIC AND ACETIC ACIDS. U. S. Pat. 1,723,298, August 6, 1929; appl. August 17, 1927.

Molasses or the hydrolysates of wood, corn cobs, etc., are fermented to lactic and acetic acids by a newly isolated organism.

(26) POLLAK, A.

PROCESS OF PROMOTING ACID FERMENTATION. U. S. Pat. 1,123,920. January 5, 1915; appl. July 15, 1913.

Lactic acid is neutralized during the fermentation with ammonium salts or hexamethylenetetramine.

(27) ———

PROCESS OF PRODUCING LACTIC ACID FROM SUGAR-CONTAINING RAW MATERIALS BY MEANS OF LACTIC-ACID BACTERIA. U. S. Pat. 1,624,674, April 12, 1927; appl. September 11, 1924.

The fermenting solution is withdrawn either continuously or intermittently and replaced with fresh carbohydrate solution.

(28) RICHTER, G. A.

PROCESS OF MANUFACTURING LACTIC ACID. U. S. Pat. 1,305,623, June 3, 1919; appl. November 2, 1917.

Hydrolyzed sawdust is fermented to lactic acid.

(29) ROBINSON, S. K.

PREPARATION OF LACTIC SALTS. U. S. Pat. 1,726,768, September 3, 1929; appl. April 24, 1925.

Whey is fermented, and the lactic acid formed is neutralized intermittently with sodium hydroxide solution as the fermentation proceeds.

(30) RÖMER, A.

FERMENTATION PROCESS. U. S. Pat. 1,510,195, September 30, 1924; appl. August 27, 1919. U. S. Pat. 1,510,196, September 30, 1924; appl. April 9, 1921.

A continuous fermentation process is described for the manufacture of various acids, including lactic, and alcohols, in which sugar solutions are passed through a series of closed fermenters.

(31) SAXE, E.

IMPROVEMENTS IN A PROCESS FOR PRODUCING LACTIC ACID FROM CERTAIN WASTE DOUGHS OF CRACKER AND BISCUIT BAKERIES. Brit. Pat. 14,479 (A. D. 1913), January 22, 1914; appl. June 23, 1913.

Scrap dough and bakery wastes are hydrolyzed and fermented to lactic acid.

(32) SAXE, S.

MANUFACTURE OF LACTIC ACID. U. S. Pat. 1,249,511, December 11, 1917; appl. April 20, 1917.

Hydrolyzed vegetable ivory nuts are subjected to a lactic fermentation.

(33) SOCIÉTÉ ANONYME DES DISTILLERIES DES DEUX-SEVRES.

IMPROVEMENTS IN AND RELATING TO THE MANUFACTURE OF ACETIC ACID AND LACTIC ACID BY FERMENTATION. Brit. Pat. 316,287, July 3, 1930; appl. April 5, 1929.

Suitable sugar solutions are fermented to lactic and acetic acids by a strain of *Lactobacillus acidophilous B.* Details of methods for the isolation of the organism are given.

(34) LA SOCIÉTÉ ANONYME DES MANUFACTURES DE PRODUITS CHIMIQUES DU NORD, AND BOULLANGER, E.

IMPROVEMENTS IN THE MANUFACTURE OF LACTIC ACID. Brit. Pat. 13,439 (A. D. 1899), June 28, 1900; appl. June 28, 1899.

A process is described in which a species of Mucor is used to hydrolyze starch and also to produce lactic acid. The mold may be used either alone or in conjunction with other lactic organisms.

(35) THOMPSON, C. O.

MANUFACTURE OF LACTIC ACID AND LACTATES. U. S. Pat. 290,284, December 18, 1883; appl. April 10, 1883, renewed November 21, 1883.

Improvement in the lactic fermentation is accomplished by digesting amylaceous material in warm water, converting part of it to glucose, and adding to the liquor pure glucose dissolved in water.

(36) WAHL, R.

PERFECTIONNEMENTS DANS LA FABRICATION D'UN LIQUIDE AIGRE OU ACIDE. Fr. Pat. 447,734, November 5, 1912; appl. September 5, 1911.

Carbohydrates are fermented by pure cultures of lactic bacteria to produce a grade of lactic acid which might be used in place of edible vinegar.

(37) WAITE, C. N.

MANUFACTURE OF LACTIC ACID. U. S. Pat. 365,655, June 28, 1887; appl. October 13, 1886.

The lactic acid fermentation of sugars is carried out in the presence of glue and neutralizing agents.

(38) ———

PROCESS OF LACTIC FERMENTATION. U. S. Pat. 368,032, August 9, 1887; appl. August 6, 1884.

Sugars and starch free from albuminoids and containing ammonium salts and phosphoric acid are fermented under anaerobic conditions to lactic acid.

(39) WHETHAM, W. E.

IMPROVEMENTS IN OR RELATING TO THE PREPARATION OR TREATMENT OF FLOUR. Brit. Pat. 18,954 (A. D. 1898), December 5, 1899; appl. September 5, 1898.

A mixture of malted barley, invert sugar, nitrogenous matter, calcium carbonate, and water is subjected to a lactic acid fermentation.

PROPIONIC ACID

(1) SHERMAN, J. M., and SHAW, R. H.

PROCESS FOR THE ACCELERATION OF PROPIONIC FERMENTATION. U. S. Pat. 1,459,959, June 26, 1923; appl. August 26, 1922.

The production of propionates and acetates from carbohydrates and salts of organic acids is accelerated by the presence of various organisms which themselves do not produce propionic acid.

(2) SHERMAN, J. M., and SHAW, R. H.
PROCESS FOR THE PRODUCTION OF PROPIONATES AND PROPIONIC ACID.
U. S. Pat. 1,470,885, October 16, 1923; appl. August 26, 1922.
Carbohydrates are fermented in the presence of calcium carbonate by the action of propionic acid forming bacteria such as *Bacillus acidi propionici*.

(3) ——— and WHITTIER, E. O.
PROCESS FOR THE PURIFICATION OF PROPIONATES BY THE ACTION OF BACTERIA. U. S. Pat. 1,450,392, April 3, 1923; appl. August 26, 1922.
Acetic acid, acetates, and other fermentable organic acids and their salts are removed from propionic acid by inoculating the solutions with proteolytic organisms such as *Bacterium vulgaris*, or acid destroying organisms, such as *Bacterium alcaligenes*, which do not attack propionic acid.

(4) WILSON, P. W. (THE COMMERCIAL SOLVENTS CORPORATION, ASSIGNEE.)
PROPIONIC ACID FERMENTATION. Canad. Pat. 302,531, July 29, 1930; appl. July 18, 1929.
Carbohydrate mashes deficient in protein are inoculated with propionic acid bacteria, and the fermentation is allowed to proceed at pH 5 to 7. Nutrient is supplied in part by yeast water or waste distillery liquor.

(5) WOODRUFF, J. W., and WILSON, P. W. (THE COMMERCIAL SOLVENTS CORPORATIONS, ASSIGNEE.)
PROPIONIC ACID FERMENTATION. Canad. Pat. 302,532, July 29, 1930; appl. July 18, 1929.
Similar to Canad. Pat. 302,531 (Reference 4).

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(4) BARY, A. DE
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